The effect of dehydroepiandrosterone on regional blood flow in prepubertal anaesthetized pigs

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Dehydroepiandrosterone has been implicated in vascular disease and its associated insulin resistance and hypertension, though little is known about its vascular effects. We have recently shown in prepubertal anaesthetized pigs that intravenous infusion of dehydroepiandrosterone caused coronary vasoconstriction through the inhibition of a vasodilatory β-adrenergic receptor-mediated effect related to the release of nitric oxide. The present study was designed to investigate the effect of dehydroepiandrosterone on mesenteric, renal and iliac vascular beds. In prepubertal pigs of both sexes anaesthetized with sodium pentobarbitone, changes in superior mesenteric, left renal and left external iliac blood flow caused by intravenous infusion of dehydroepiandrosterone were assessed using electromagnetic flowmeters. Changes in heart rate and arterial blood pressure were prevented by atrial pacing and by connecting the arterial system to a pressurized reservoir containing Ringer solution. In 22 pigs, infusion of 1 mg h^{-1} of dehydroepiandrosterone decreased mesenteric, renal and iliac blood flow. In a further 10 pigs, dose–response curves were obtained by graded increases in the infused dose of hormone between 0.03 and 4 mg h^{-1}. The mechanisms of the above response were studied in the 22 pigs by repeating the experiment after haemodynamic variables had returned to the control values observed before infusion. Blockade of α-adrenoceptors with intravenous phentolamine (five pigs) did not affect the dehydroepiandrosterone-induced mesenteric, renal and iliac vasoconstriction. This response was abolished by blockade of β_2-adrenoceptors with intravenous butoxamine (five pigs) and by blockade of mesenteric, renal and iliac nitric oxide synthase with intra-arterial administration of N^{ω}-nitro-L-arginine methyl ester (seven pigs), even after reversing the increase in local vascular resistance caused by the two blocking agents with intravenous infusion of papaverine. In five pigs, the increase in measured blood flow caused by intravenous infusion of isoproterenol (isoprenaline) was significantly reduced by infusion of dehydroepiandrosterone. The present study showed that intravenous infusion of dehydroepiandrosterone primarily caused mesenteric, renal and iliac vasoconstriction. The mechanisms of this response were shown to be due to the inhibition of a vasodilatory β_2-adrenergic receptor-mediated effect, which possibly involved the release of nitric oxide.

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Dehydroepiandrosterone is a principal C-19 adrenal steroid whose plasma levels significantly increase following puberty, but decline with advancing age (Parker, 1999). There has been a controversy as to whether or not this hormone is involved in the development of vascular disease (LaCroix *et al.* 1992; Nestler *et al.* 1992; Herrington, 1995; Alexandersen *et al.* 1996; Khaw, 1996; Porsova-Dutoit *et al.* 2000), insulin resistance, obesity and hypertension (Nestler *et al.* 1992; Ebeling & Koivisto, 1994; Barrett-Connor & Goodman-Gruen, 1995*a*,*b*; Fuenmayor *et al.* 1997; Suzuki *et al.* 1999; Kroboth *et al.* 1999; Mino *et al.* 2002; Saruc *et al.* 2003; Kawano *et al.* 2003).

We have recently shown in prepubertal anaesthetized pigs that the acute administration of this steroid hormone causes coronary vasoconstriction, the mechanism of which involved the inhibition of a vasodilatory β-adrenergic effect related to the release of nitric oxide (Molinari *et al.* 2003). In contrast, in the same experimental model, the acute effect of other steroid hormones such as 17β-oestradiol, progesterone and testosterone caused

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vasodilatation in the coronary, mesenteric, renal and iliac regions through mechanisms which involved the release of nitric oxide (Vacca *et al.* 1999; Molinari *et al.* 2001*a*,*b*, 2002). In these studies, there were marked differences between the regions in the magnitude of vascular dilatation, which was greater in the case of the coronary circulation, and it is as yet unknown whether such regional differences apply to the acute regional vascular effect of dehydroepiandrosterone. Indeed, there has been very little information on the effect of dehydroepiandrosterone on peripheral vascular beds. A dose-dependent relaxation of precontracted isolated helical strips of rat tail artery caused by dehydroepiandrosterone-induced modulation of the intracellular calcium metabolism has been described (Barbagallo *et al.* 1995), but there is no available information on the *in vivo* acute effects of this hormone on other regional vascular beds.

The present work was therefore planned to study the primary *in vivo* effects of the acute administration of dehydroepiandrosterone on mesenteric, renal and iliac blood flow in prepubertal pigs of both sexes and to determine the mechanisms involved. This was achieved by intravenously infusing the hormone whilst preventing changes in heart rate and arterial blood pressure to avoid secondary interference by reflex and local physical effects. In addition, a dose–response study was also performed.

Methods

The experiments were carried out in 32 prepubertal pigs, weighing 73–80 kg, supplied by an accredited dealer (Azienda Cornelia srl, San Pietro Mosezzo, Novara, Italy). The age of the pigs was less than 5 months and 16 of them were male. The animals were fasted overnight and then anaesthetized with intramuscular ketamine ($20\ mg\ kg^{-1}$; Parke-Davis, Milan, Italy) followed after about 15 min by intravenous sodium pentobarbitone ($15\ mg\ kg^{-1}$; Siegfried, Zofingen, Switzerland), after which they were artificially ventilated with oxygen-enriched air using a respiratory pump (Harvard 613; Harvard Apparatus, South Natick, MA, USA). Anaesthesia was maintained throughout the experiments by continuous I.V. infusion of sodium pentobarbitone ($7\ mg\ kg^{-1}\ h^{-1}$) and assessed as previously reported (Linden & Mary, 1983) from responses of the animals to somatic stimuli. The experiments were carried out in accordance with national guidelines (D.L.G.S. 27/01/1992, no. 116).

Pressures in the ascending aorta and in the right atrium were recorded via catheters connected to pressure transducers (Statham P23 XL; Gould, Valley View, OH, USA) inserted into the right femoral artery and the right external jugular vein, respectively. The abdomen was opened through a mid-line incision and an electromagnetic flowmeter probe (model BL 613; Biotronex Laboratory Inc., Chester, MD, USA) was positioned near the origin of the superior mesenteric, left renal and left external iliac arteries. Distal to the probe a plastic snare was placed around each artery for zero blood flow assessment. Each probe was calibrated *in vitro* at the end of each experiment.

To prevent changes in arterial blood pressure during the experiments, the chest was opened in the left fourth intercostal space and a large-bore cannula was introduced into the left internal mammary artery and connected to a reservoir containing Ringer solution (SIFRA-Società Italiana Farmaceutici Ravizza, Verona, Italy) kept at 38°C. The reservoir was pressurized using compressed air, which was controlled with a Starling resistance, and pressure within the reservoir was measured by a mercury manometer. This method has been shown in anaesthetized pigs to allow the arterial blood pressure to be maintained at steady levels without significant changes in filling pressures of the heart or the haematocrit (e.g. Vacca *et al.* 1999; Molinari *et al.* 2003). Coagulation of the blood was avoided by the intravenous injection of heparin (Parke Davis; initial doses of 500 i.u. kg^{-1}, and subsequent doses of 50 i.u. kg^{-1} every 30 min).

To pace the heart, electrodes were sewn on the left atrial appendage and connected to a stimulator (model S8800; Grass Instruments, Quincy, MA, USA) which delivered pulses of 3–5 V with 2 ms durations at the required frequency. Arterial blood samples were used to measure pH, arterial partial pressures of oxygen and carbon dioxide (P_{O_2} and P_{CO_2}) (with a gas analyser; IL 1304; Instrumentation Laboratory, Lexington, MA, USA) and the haematocrit. Normal values of pH, P_{O_2} and P_{CO_2} of 7.42 ± 0.02, 84.3 ± 3.9 mmHg and 39.8 ± 0.7 mmHg, respectively, have been reported in prepubertal pigs (Houpt, 1986). In the present study, the animals were artificially ventilated with oxygen-enriched air and values of pH and P_{CO_2} were maintained within normal limits during the experiments by the intravenous infusion of a solution of 2.8% sodium bicarbonate and by adjusting the respiratory stroke volume, when necessary (Linden & Mary, 1983). The rectal temperature of the pigs was monitored and kept between 38 and 40°C using an electric pad.

Mean aortic and right atrial pressures and mean and phasic mesenteric, renal and iliac blood flows were monitored and recorded together with heart rate by using an electrostatic strip-chart recorder (Gould ES 2000). The heart rate was obtained from the electrocardiogram with

a ratemeter (ECG/Biotach amplifier, model 13-4615-65 A; Gould).

Regional vascular resistance in the mesenteric, renal and iliac circulations was calculated as the ratio between values of mean aortic blood pressure and mean blood flow.

In some pigs, the serum concentration of dehydroepiandrosterone was measured during the experiments by radioimmunoassay, using IM1138 Immunotech kit (Beckman Coulter Inc., CA, USA). In the same experiments, serum concentrations of dehydroepiandrosterone sulphate, testosterone and 17β-oestradiol were measured by immunoassay, using L2KDS2, L2KTT2 and L2KE22 Immulite 2000 kits (DPC, Diagnostic Products Corp., CA, USA), respectively. For each measurement, 2 ml venous blood samples were withdrawn from the right femoral vein and replaced with an equal volume of Ringer solution. Serum obtained from each sample was split to provide duplicates before being stored at -20°C for subsequent measurements. Analytical sensitivity, determined as the concentration equivalent to the difference between mean counts obtained from the zero sample and two standard deviations for competitive assays, was 0.3 ng ml^{-1} for dehydroepiandrosterone, 14 ng ml^{-1} for dehydroepiandrosterone sulphate, 0.2 ng ml^{-1} for testosterone and 15 pg ml^{-1} for 17β-oestradiol. The variability of measurement was assessed by determining individual differences between duplicate samples assayed blind.

At the end of the experiment, each animal was killed by an intravenous injection of 90 mg kg^{-1} sodium pentobarbitone.

Experimental protocol

The experiments were begun after at least 30 min of steady-state conditions with respect to measured haemodynamic variables. In the 32 pigs, to avoid the interference of any possible changes in heart rate and arterial blood pressure during the experiments, the heart was paced to a frequency higher, by 20 beats min^{-1}, than that observed during the steady state and the arterial system was connected to the pressurized reservoir. After at least 10 min of steady-state conditions, the experiments were carried out by intravenously infusing either saline only or a solution of 1 mg of dehydroepiandrosterone (Sigma) in 2 ml of serum diluted in saline, in random order. The infusions were completed within a period of 1 h by using an infusion pump (Model 22; Harvard Apparatus) working at constant rate of 1 ml min^{-1}. After the infusion was stopped, observations were continued for 30 min. The dose of dehydroepiandrosterone used corresponded to that given to elderly subjects to restore their circulating levels to those of young adults (Legrain *et al.* 2000) and was the same as previously used in anaesthetized pigs to study the coronary effects of the hormone (Molinari *et al.* 2003).

Recordings taken for 10 min during the steady state before infusion of dehydroepiandrosterone were used as control. Measurements of haemodynamic variables were obtained during the last 10 min of infusion in the steady state and compared with control values. The effect of infusion of 1 mg h^{-1} of dehydroepiandrosterone on mesenteric, renal and iliac blood flow and the mechanisms involved were studied in 22 pigs (11 male). In the remaining 10 pigs (five male), a dose–response study was carried out by gradually increasing the infused dose of dehydroepiandrosterone in 12 steps from a minimum value of 0.03 mg h^{-1} to a maximum value of 4 mg h^{-1}. Each dose was infused for 20 min. The resulting changes in mesenteric, renal and iliac blood flow were compared with control values obtained before starting the infusion.

The mechanisms of the response of mesenteric, renal and iliac blood flow to the infusion of dehydroepiandrosterone were studied in the group of 22 pigs by repeating the experiment after haemodynamic variables had returned to control levels. In five pigs, dehydroepiandrosterone was administered after blockade of α-adrenoceptors with intravenous administration of phentolamine (1 mg kg^{-1}; Ciba Geigy, Varese, Italy) and in five pigs after blockade of β_2-adrenoceptors with intravenous administration of butoxamine (2.5 mg kg^{-1}; Sigma). In five pigs, the effect of 5 min intravenous infusion of isoproterenol (0.05 μg kg^{-1} min^{-1}) on baseline values of heart rate at constant arterial blood pressure was assessed. After haemodynamic variables had returned to control values (within 10 min), the isoproterenol infusion at constant arterial blood pressure was repeated whilst pacing the heart to a frequency higher, by 20 beats min^{-1}, than that observed during the previous infusion of isoproterenol to assess the increases in mesenteric, renal and iliac blood flow caused by the β-adrenergic agent. After blood flow had returned to control levels, the effect of infusion of dehydroepiandrosterone on the isoproterenol-induced blood flow increases was assessed by repeating the infusion of the β-adrenergic agent during the last 5 min of the hormone infusion. In the remaining seven pigs, dehydroepiandrosterone was administered after blockade of nitric oxide synthase with intra-arterial administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME; Sigma). In these seven pigs, intra-arterial administration of

L-NAME in each of the three arteries was performed at a dose of 2 mg for ml min^{-1} of measured blood flow during the control period. All the drugs were given in the absence of pacing of the heart and without controlling arterial blood pressure and their effects on haemodynamic variables were measured in the steady state. In all subsequent experiments, changes in heart rate and arterial blood pressure were prevented. In five of the seven pigs which received intra-arterial L-NAME, dehydroepiandrosterone was infused for 30 min firstly after injecting the blocking agent into the mesenteric artery. The infusion of the hormone was then repeated after injecting L-NAME into the renal artery and, finally, after injecting L-NAME into the iliac artery. In the remaining two pigs, the infusion of the hormone was performed after injecting L-NAME into all the three arteries. In these two pigs and in two of the butoxamine-treated pigs, the infusion of dehydroepiandrosterone was performed when a steady state was attained during a continuous intravenous infusion of papaverine (Sigma) at a dose of 3.5–4.5 mg kg^{-1} h^{-1}. This procedure was used to reverse the increases in regional vascular resistance caused by the two blocking agents.

Student's paired *t* test was used to examine changes in measured variables caused by dehydroepiandrosterone infusion. Student's unpaired *t* test was used to compare the effects of dehydroepiandrosterone infusion on measured blood flows in male and female pigs. A value of $P < 0.05$ was considered statistically significant. Group data are presented as means ± S.D. (range).

Results

In all pigs, recordings commenced approximately 5 h after the induction of anaesthesia. The mean (range) pH, P_{O_2} and P_{CO_2} of arterial blood were, respectively, 7.40 ± 0.01 (7.38–7.43), 116.6 ± 8.8 (104–137) mmHg and 39.7 ± 1 (38–41) mmHg and the haematocrit was 37.4 ± 1.2 (36–40)%.

Effects of infusion of dehydroepiandrosterone

In five of the group of 22 pigs, values of serum levels of dehydroepiandrosterone, 17β-oestradiol and testosterone in the control before infusion were, respectively, 0.6 ± 0.3 (0.3–1) ng ml^{-1}, 37.4 ± 6.4 (29.8–43.8) pg ml^{-1} and 2.2 ± 0.5 (1.6–2.8) ng ml^{-1}. Infusion of dehydroepiandrosterone caused an increase of its serum levels of 3.2 ± 0.6 (2.6–4.1) ng ml^{-1} ($P < 0.0005$). Changes in serum levels of 17β-oestradiol and testosterone were not significant ($P > 0.45$ and $P > 0.30$, respectively). Serum levels of dehydroepiandrosterone sulphate were not detectable, being smaller than the threshold of measurement.

In the group of 22 pigs, infusion of the vehicle (60 ml of saline) did not cause changes in the control values of measured haemodynamic variables. Group values of data and individual changes in mean mesenteric, renal and iliac blood flow caused by infusion of dehydroepiandrosterone are shown in Table 1 and Fig. 1, respectively. In each pig, infusion of dehydroepiandrosterone caused a decrease in mean mesenteric, renal and iliac blood flow. Group decreases in these flows, respectively, amounted to −9.8 ± 2.9 (−16.9 to −4.6)%, −10 ± 2 (−17.4 to −7.2)% and −11.4 ± 2.7 (−16.5 to −6.7)% of the control values and corresponded to increases in mesenteric, renal and iliac vascular resistance of 11 ± 3.6 (4.5–19.7)%, 11.1 ± 4.3 (6.3–27.3)% and 13.3 ± 3.1 (9.1–20)% from control values of 0.094 ± 0.021 (0.071–0.138), 0.20 ± 0.03 (0.15–0.27) and 0.87 ± 0.20 (0.33–1.16) mmHg ml^{-1} min^{-1}. Group decreases in measured blood flows obtained in the 11 male and 11 female pigs of this group were, respectively, −91 ± 31 (−150 to −47) ml min^{-1} ($P < 0.0005$) and −104 ± 38 (−205 to −60) ml min^{-1} ($P < 0.0005$) from control values of 993 ± 186 (740–1280) and 994 ± 166 (776–1313) ml min^{-1} for mesenteric blood flow, −44 ± 7 (−56 to −34) ml min^{-1} ($P < 0.0005$) and −47 ± 12 (−78 to −33) ml min^{-1} ($P < 0.0005$) from control values of 461 ± 82 (367–617) and 449 ± 46 (379–501) ml min^{-1} for renal blood flow, and −12 ± 3 (−19 to −8) ml min^{-1} ($P < 0.0005$) and −13 ± 4 (−24 to −9) ml min^{-1} ($P < 0.0005$) from control values of 113 ± 36 (89–210) and 108 ± 23 (85–156) ml min^{-1} for iliac blood flow. The differences in the responses of measured blood flow between male and female pigs were not significant ($P > 0.35$, $P > 0.30$ and $P > 0.30$ for mesenteric, renal and iliac flow, respectively). Changes in mean right atrial pressure during these experiments were not significant (Table 1). An example of the above response is shown in Fig. 2. In the group of 22 pigs, the effect of dehydroepiandrosterone began within about 3 min after starting the infusion and reached a steady state in about 5 min. Measured blood flows returned to control values within 5 min after the end of the infusion. These results indicate that infusion of dehydroepiandrosterone causes mesenteric, renal and iliac vasoconstriction.

Dose–response study

Serum levels of dehydroepiandrosterone, 17β-oestradiol and testosterone in the control before infusions were, respectively, 0.6 ± 0.2 (0.4–0.9) ng ml^{-1}, 36 ± 5.9 (28.3–41.3) pg ml^{-1} and 1.9 ± 0.3 (1.5–2.3) ng ml^{-1}. Infusions

Table 1. Changes in haemodynamic variables caused by intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone in 22 pigs

Data	Control	Test	Change
HR (beats min^{-1})	113.1 ± 11.6 (94–131)	113.1 ± 11.5 (94–131.2)	0.03 ± 0.1 (−0.2 to 0.3)
ABP (mmHg))	90.8 ± 9.6 (70–110)	91 ± 9.6 (70–110)	0.2 ± 0.6 (−1 to 2)
RAP (mmHg)	3.36 ± 0.6 (2.2–4.1)	3.33 ± 0.5 (2.2–4.1)	−0.03 ± 0.1 (−0.2 to 0.3)
MBF (ml min^{-1})	993 ± 172 (740–1313)	896 ± 162 (665–1253)	−97 ± 35 (−205 to −47)*
RBF (ml min^{-1})	455 ± 65 (367–617)	409 ± 62 (326–565)	−46 ± 10 (−78 to −33)*
IBF (ml min^{-1})	111 ± 30 (85–210)	98 ± 28 (71–196)	−13 ± 4 (−24 to −8)*

Data are means ± S.D. (range). HR, heart rate; ABP, mean aortic blood pressure; RAP, mean right atrial pressure; MBF, mean mesenteric blood flow; RBF, mean renal blood flow; IBF, mean iliac blood flow. *$P < 0.0005$ *versus* control

of dehydroepiandrosterone caused a progressive increase in its serum levels (Fig. 3). Changes in serum levels of 17β-oestradiol and testosterone were not significant (at least $P > 0.05$ and $P > 0.10$, respectively). Serum levels of dehydroepiandrosterone sulphate were not detectable.

In the group of 10 pigs, control values of mean mesenteric, renal and iliac blood flow were, respectively, 916 ± 102 (786–1139), 477 ± 56 (387–600) and 106 ± 19 (85–142) ml min^{-1}. The results obtained, which indicate the effects of accumulating doses of dehydroepiandrosterone, are shown in Fig. 4. The threshold dose of the hormone was found to be between 0.05 and 0.1 mg h^{-1}. In three of the 10 pigs, the threshold dose for renal blood flow was between 0.03 and 0.05 mg h^{-1}. Maximal effects for all measured blood flows were observed at a dose of 3 mg h^{-1}. Analysis of the responses of mesenteric, renal and iliac blood flow to each dose of dehydroepiandrosterone showed that there were no significant differences between male and female pigs (at least $P > 0.05$, $P > 0.20$ and $P > 0.10$, respectively).

Experiments after blockade of α-adrenoceptors

The administration of phentolamine in five pigs caused a decrease in mean aortic blood pressure of −16.8 ± 2.8 (−20 to −13) mmHg ($P < 0.0005$) from control values of 91.2 ± 5.7 (85−100) mmHg and an increase in heart rate of 11 ± 2.6 (9−15) beats min^{-1} ($P < 0.0005$) from control values of 102.4 ± 9.8 (87–111) beats min^{-1}. These changes were accompanied by small group decreases in mesenteric, renal and iliac blood flow of −51 ± 64 (−125 to 50) ml min^{-1} ($P > 0.05$), −48 ± 51 (−122 to 3) ml min^{-1} ($P > 0.05$) and −3 ± 9 (−15 to 9) ml min^{-1} ($P > 0.20$) from control values of 993 ± 248 (770–1310), 423 ± 66 (363–497) and 96 ± 9 (84–106) ml min^{-1}, respectively.

Blockade of α-adrenoceptors did not affect the dehydroepiandrosterone-induced decreases in mesenteric, renal and iliac blood flow. Group decreases in these flows were, respectively, –103 ± 56 (−198 to −62) ml min^{-1} ($P < 0.01$), −41 ± 7 (−50 to −32) ml min^{-1} ($P < 0.0005$) and −10 ± 1 (−11 to −9) ml min^{-1} ($P < 0.0005$). During these experiments, changes in mean right atrial pressure were not significant ($P > 0.15$). In the same five pigs, the decreases in mesenteric, renal and iliac blood flow obtained with infusion of dehydroepiandrosterone before blockade of α-adrenoceptors, respectively, were −106 ± 58 (−205 to −60) ml min^{-1} ($P < 0.01$), −41 ± 8 (−53 to −33) ml min^{-1} ($P < 0.0005$) and −12 ± 2 (−14 to −9) ml min^{-1} ($P < 0.0005$) ml min^{-1}. The differences between the responses before and after blockade were not significant ($P > 0.10$, $P > 0.20$ and $P > 0.10$ for mesenteric, renal and iliac flow, respectively). A comparison between individual responses of mesenteric, renal and iliac blood flow before and after blockade of α-adrenoceptors is shown in Fig. 5.

Experiments after blockade of β_2-adrenoceptors

The administration of butoxamine in five pigs caused an increase in mean aortic blood pressure of 11.2 ± 3.6 (8−17) mmHg ($P < 0.0025$) from control values of 90.4 ± 14.5 (70–108) mmHg and a decrease in heart rate of –8 ± 2.7 (−12 to −6) beats min^{-1} ($P < 0.0025$) from control values of 101 ± 10.2 (88−115) beats min^{-1}. These changes were accompanied by group decreases in mesenteric, renal and iliac blood flow of −60 ± 62 (−161 to 9) ml min^{-1} ($P < 0.05$), −37 ± 38 (−99 to 2) ml min^{-1} ($P < 0.05$) and −6 ± 5 (−14 to 1) ml min^{-1} ($P < 0.05$) from control values of 967 ± 128 (936–1180), 467 ± 86 (390–612) and 94 ± 6 (86–100) ml min^{-1}, respectively. In two of these pigs, infusion of papaverine decreased mesenteric vascular resistance by −0.027 and −0.015 mmHg ml^{-1} min^{-1}, renal vascular resistance by −0.04 and −0.04 mmHg ml^{-1} min^{-1}, and iliac vascular

resistance by −0.24 and −0.28 mmHg ml^{-1} min^{-1}. In the same pigs, the increases in mesenteric, renal and iliac vascular resistance caused by butoxamine were, respectively, 0.030 and 0.011 mmHg ml^{-1} min^{-1}, 0.03 and 0.05 mmHg ml^{-1} min^{-1}, and 0.17 and 0.28 mmHg ml^{-1} min^{-1}.

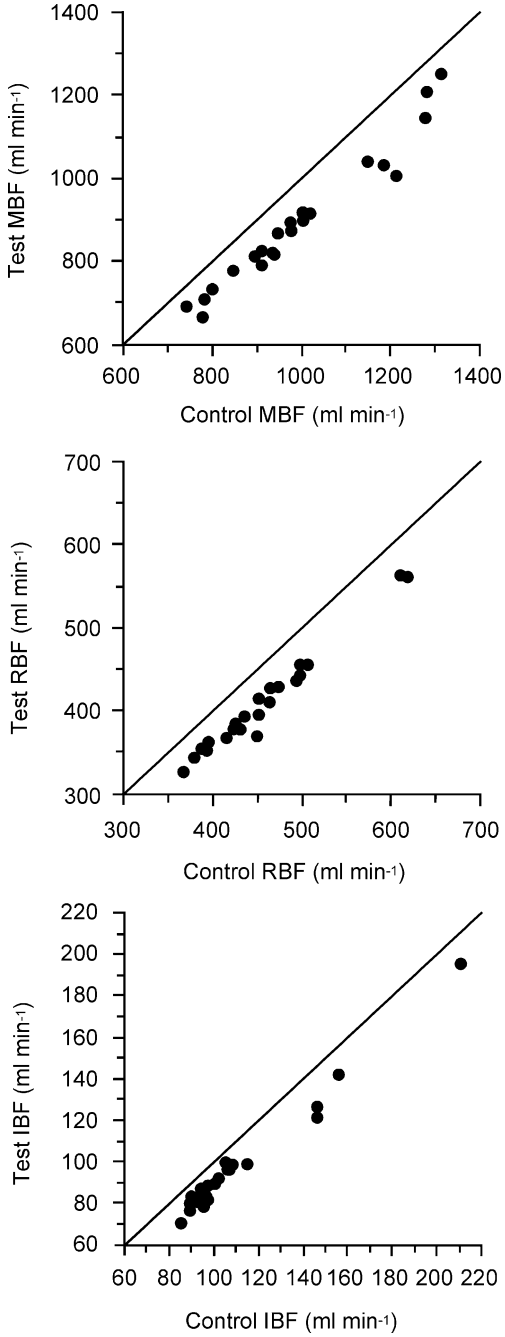

Figure 1. The response of mean mesenteric blood flow (MBF), mean renal blood flow (RBF) and mean iliac blood flow (IBF) to the intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone in 22 pigs

The values of blood flow obtained during the test period of measurement are plotted against the corresponding control values before infusion. The continuous line is the line of equality.

In each of the five treated pigs, blockade of β_2-adrenoceptors completely prevented the decreases of mesenteric, renal and iliac blood flow caused by dehydroepiandrosterone (Fig. 6). During the test period of measurement, changes in these flows were small and insignificant, amounting to, respectively, -0.4 ± 5 (-7–6) ml min^{-1} ($P > 0.40$), -1 ± 3 (-5–2) ml min^{-1} ($P > 0.20$) and 1 ± 2 (-1–3) ml min^{-1} ($P > 0.15$). During these experiments, changes in mean right atrial pressure were not significant ($P > 0.10$). In the same five pigs, the decreases in mesenteric, renal and iliac blood flow obtained with infusion of dehydroepiandrosterone before blockade of β_2-adrenoceptors were, respectively, -106 ± 34 (-150 to -66) ml min^{-1} ($P < 0.0025$), -44 ± 9 (-56 to -34) ml min^{-1} ($P < 0.0005$) and -11 ± 2 (-15 to -9) ml min^{-1} ($P < 0.0005$). These results indicate that the mechanisms of the mesenteric, renal and iliac vasoconstriction caused by infusion of dehydroepiandrosterone involve β_2-adrenoceptors.

Experiments with isoproterenol

In the five pigs treated, a preliminary infusion of isoproterenol at constant arterial blood pressure caused an increase in heart rate of 37.4 ± 3.5 (33–42) beats min^{-1} ($P < 0.0005$) from control values of 85.4 ± 7.9 (76–95) beats min^{-1}.

Infusion of the β-adrenergic agent at constant heart rate and arterial blood pressure caused increases in mesenteric, renal and iliac blood flow of 336 ± 58 (250–398) ml min^{-1} ($P < 0.0005$), 126 ± 23 (111–166) ml min^{-1} ($P < 0.0005$) and 28 ± 4 (23–33) ml min^{-1} ($P < 0.0005$), respectively, from control values of 999 ± 173 (800–1275), 450 ± 43 (393–501) and 100 ± 9 (91–113) ml min^{-1}. Subsequent infusion of dehydroepiandrosterone decreased mesenteric, renal and iliac blood flow by -95 ± 25 (-134 to -69) ml min^{-1} ($P < 0.0025$), -43 ± 5 (-49 to -34) ml min^{-1} ($P < 0.0005$) and -12 ± 3 (-16 to -9) ml min^{-1} ($P < 0.0005$). The differences between these decreases and those obtained during the previous infusion of the hormone in the same animals were not significant ($P > 0.40$, $P > 0.40$ and $P > 0.25$ for mesenteric, renal and iliac flow, respectively). Infusion of isoproterenol during the last 5 min of dehydroepiandrosterone infusion caused increases in mesenteric, renal and iliac blood flow of 140 ± 20 (115–167) ml min^{-1} ($P < 0.0005$), 60 ± 8 (51–69) ml min^{-1} ($P < 0.0005$) and 13 ± 3 (10–16) ml min^{-1} ($P < 0.0005$),

respectively. The responses of these flows to isoproterenol infusion were decreased by dehydroepiandrosterone by -53.6 ± 6.7 (-64.9 to -48.6)% ($P < 0.0005$), 47.3 ± 6.7 (-54.1 to -40.3)% ($P < 0.0005$) and -49 ± 6.4 (-56.2 to -39)% ($P < 0.0005$). These results confirmed that the mechanisms of the mesenteric, renal and iliac vasoconstriction caused by infusion of dehydroepiandrosterone involve β_2-adrenoceptors.

Role of nitric oxide

In the seven pigs treated, spontaneous control values of heart rate and mean arterial blood pressure before the administration of L-NAME were 87.3 ± 6.4 (76–98) beats min^{-1} and 88.9 ± 10.4 (75–98) mmHg. Intra-arterial injections of L-NAME caused increases in these variables of 7.1 ± 4.6 (-2–12) beats min^{-1} ($P < 0.005$) and 22.1 ± 8.6 (11–35) mmHg ($P < 0.0005$). Injection of the blocking agent into the mesenteric artery caused a group decrease in mesenteric blood flow of -28 ± 45 (-83–27) ml min^{-1} ($P > 0.05$) from a control value of 1000 ± 172 (745–1275) ml min^{-1}. Injection of L-NAME into the renal artery caused a decrease in renal blood flow of -26 ± 18 (-50 to -7) ml min^{-1} ($P < 0.005$) from a control value of 465 ± 65 (410–602) ml min^{-1}. Injection of L-NAME into the iliac artery caused a group decrease in iliac blood flow of -7 ± 7 (-21–2) ml min^{-1} ($P < 0.025$) from a control value of 134 ± 39 (90–205) ml min^{-1}. In the two pigs in which L-NAME was injected in all three arteries, infusion of papaverine

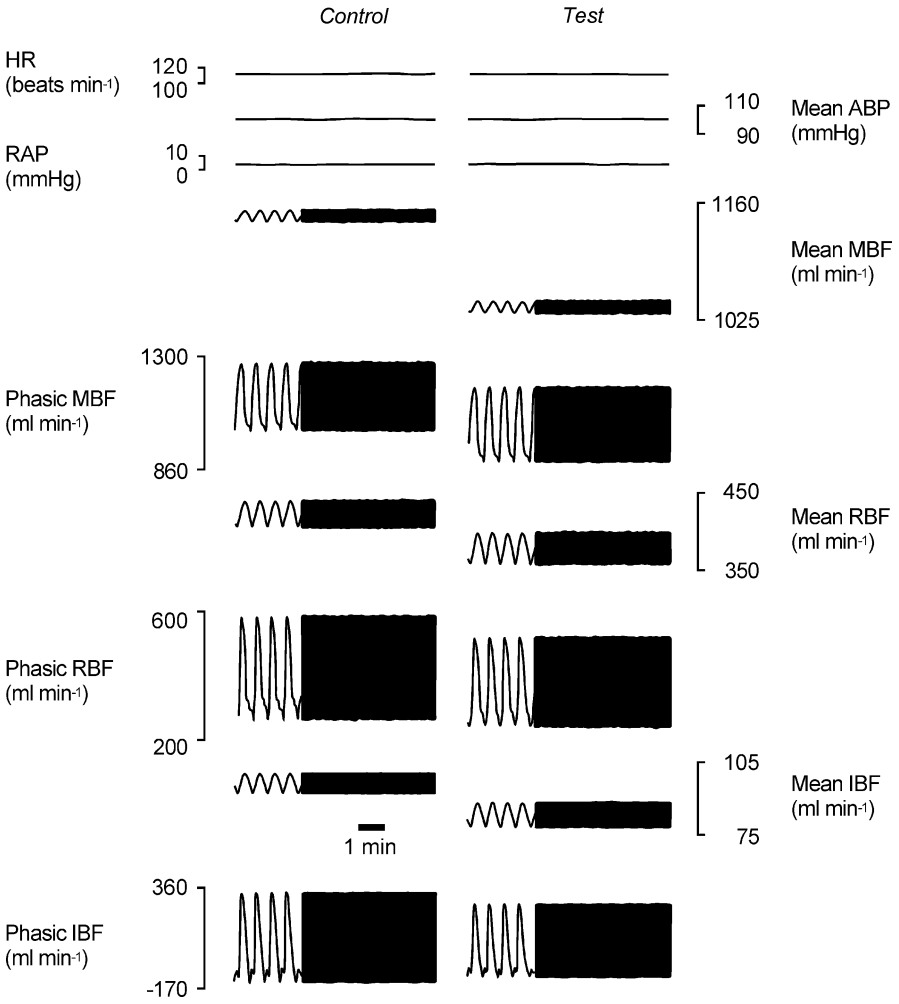

Figure 2. Example of experimental recordings showing the haemodynamic effects of the intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone in one pig

From top to bottom, traces show heart rate (HR), mean aortic blood pressure (ABP), mean right atrial pressure (RAP), mean and phasic mesenteric blood flow (MBF), mean and phasic renal blood flow (RBF), mean and phasic iliac blood flow (IBF).

decreased mesenteric vascular resistance by −0.029 and −0.030 mmHg ml^{-1} min^{-1}, renal vascular resistance by −0.09 and −0.08 mmHg ml^{-1} min^{-1}, and iliac vascular resistance by −0.43 and −0.37 mmHg ml^{-1} min^{-1}. In the same pigs, the increases in mesenteric, renal and iliac vascular resistance caused by L-NAME were, respectively, 0.026 and 0.025 mmHg ml^{-1} min^{-1}, 0.08 and 0.07 mmHg ml^{-1} min^{-1}, and 0.39 and 0.35 mmHg ml^{-1} min^{-1}.

L-NAME injection into the mesenteric artery. In each of the five pigs treated, blockade of mesenteric nitric oxide synthase abolished the response of mesenteric blood flow to the infusion of dehydroepiandrosterone (Fig. 7) without affecting the responses of renal and iliac blood flow. Changes in mesenteric blood flow caused by the hormone were 1.2 ± 5.9 (−7 to 7) ml min^{-1} ($P > 0.30$) from a control value of 900 ± 112 (763–1068) ml min^{-1}. In the same five pigs, the decrease in mesenteric blood flow obtained with infusion of dehydroepiandrosterone before blockade of mesenteric nitric oxide synthase was -89 ± 26 (−112 to −47) ml min^{-1} ($P < 0.0025$). The differences between the responses of renal and iliac blood flow to dehydroepiandrosterone before and after injection of L-NAME into the mesenteric artery were not significant ($P > 0.25$ and $P > 0.20$, respectively).

L-NAME injection into the renal artery. In each of the five pigs, the subsequent blockade of renal nitric oxide synthase abolished the response of renal blood flow to the infusion of dehydroepiandrosterone (Fig. 7) without affecting the response of iliac blood flow. Changes in renal blood flow caused by the hormone were -0.8 ± 4.7 (−4 to 6) ml min^{-1} ($P > 0.35$) from a control value of 450 ± 65 (391–560) ml min^{-1}. In the same five pigs, the decrease in renal blood flow obtained with infusion of

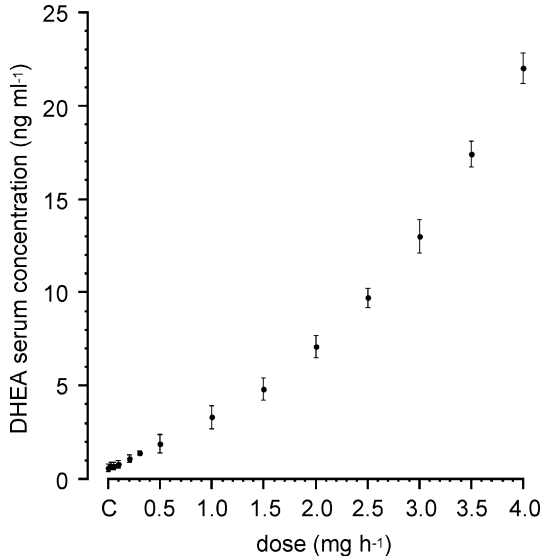

Figure 3. Serum levels of dehydroepiandrosterone (DHEA) obtained during graded increases in the infused dose of the hormone in five pigs
The means of dehydroepiandrosterone serum levels obtained during the last minute of each infusion are plotted against the dose. C, control value before infusions. The bars indicate S.D.

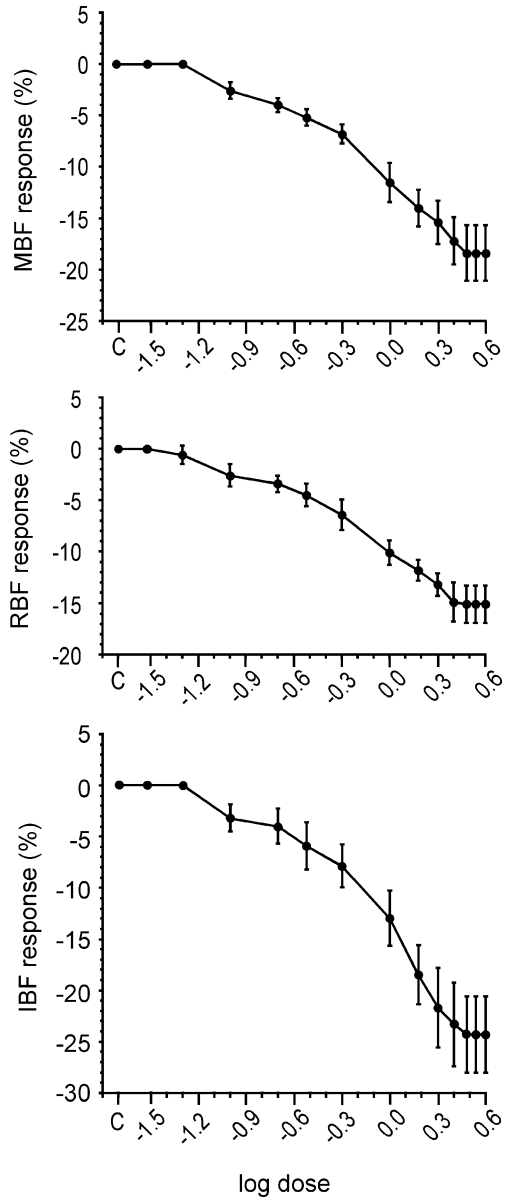

Figure 4. Responses of mean MBF, mean RBF and mean IBF to graded increases in the infused dose of dehydroepiandrosterone between 0.03 and 4 mg h^{-1} in 10 pigs
The means of percentage changes in blood flow obtained during the test period of measurement are plotted against the logarithm of the doses. C, control value before infusions. The bars indicate S.D.

dehydroepiandrosterone before blockade of renal nitric oxide synthase was -55 ± 12 (-75 to -46) ml min^{-1} ($P < 0.0005$). The difference between the response of iliac blood flow to dehydroepiandrosterone before and after injection of L-NAME into the renal artery was not significant ($P > 0.20$).

L-NAME injection into the iliac artery. In each of the five pigs, the subsequent blockade of iliac nitric oxide synthase abolished the response of iliac blood flow to the infusion of dehydroepiandrosterone (Fig. 7). Changes in iliac blood flow caused by the hormone were 0.6 ± 1.3 (-1 to 2) ml min^{-1} ($P > 0.15$) from a control value of 143 ± 27 (107–183) ml min^{-1}. In the same five pigs, the decrease in iliac blood flow obtained with infusion of dehydroepiandrosterone before blockade of iliac nitric oxide synthase was -17 ± 4 (-24 to -14) ml min^{-1} ($P < 0.0005$).

L-NAME injection into the mesenteric, renal and iliac arteries. In the two pigs examined, control values of mesenteric, renal and iliac blood flow after injection of L-NAME into the mesenteric, renal and iliac arteries and during the continuous infusion of papaverine were, respectively, 1353 and 1105 ml min^{-1}, 451 and 449 ml min^{-1} and 102 and 95 ml min^{-1}. Blockade of nitric oxide synthase abolished the responses of mesenteric, renal and iliac blood flow to the infusion of dehydroepiandrosterone. Changes in these flows caused by the hormone were, respectively, 9 and -5 ml min^{-1}, 1 and 0 ml min^{-1}, and 0 and 4 ml min^{-1}.

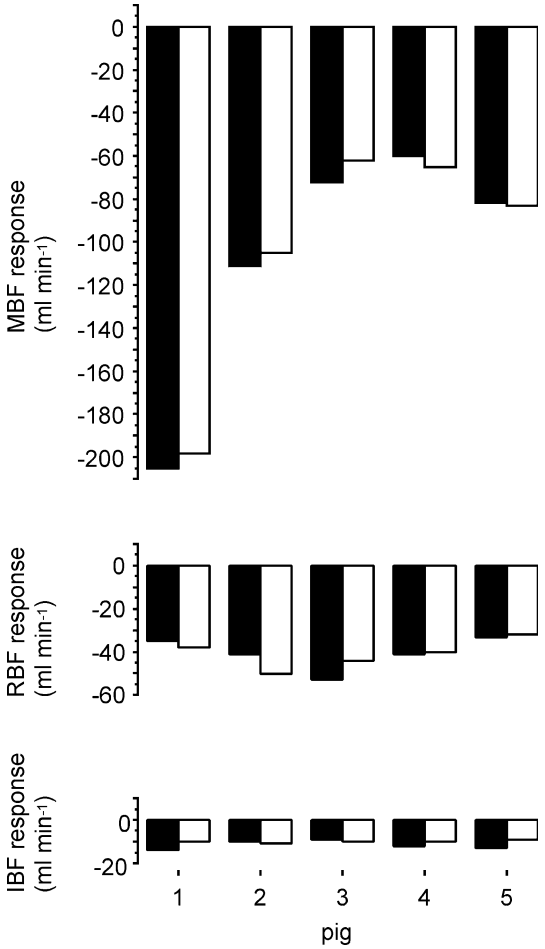

Figure 5. The effect of blockade of α-adrenoceptors on responses of mean MBF, mean RBF and mean IBF to the intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone
Dehydroepiandrosterone was infused before (filled columns) and after (open columns) blockade of α-adrenoceptors in five pigs (numbered 1–5).

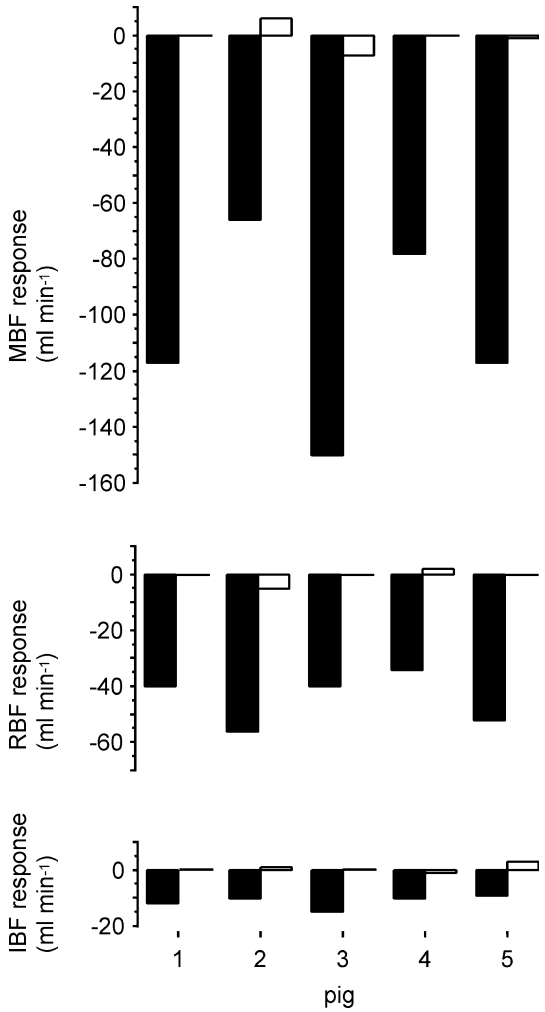

Figure 6. The effect of blockade of β_2-adrenoceptors on responses of mean MBF, mean RBF and mean IBF to the intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone
Dehydroepiandrosterone was infused before (filled columns) and after (open columns) blockade of β_2-adrenoceptors in five pigs (numbered 1–5).

These results, together with the previous data, indicate that the β_2-adrenergic receptor-mediated mesenteric, renal and iliac vasoconstriction caused by infusion of dehydroepiandrosterone may possibly involve the release of nitric oxide.

Discussion

The present investigation has shown for the first time that intravenous infusion of dehydroepiandrosterone caused decreases in blood flow and increases in vascular resistance in the mesenteric, renal and iliac regions. The mechanisms of these responses were shown to involve β_2-adrenergic receptor-mediated effects, possibly related to the release of nitric oxide.

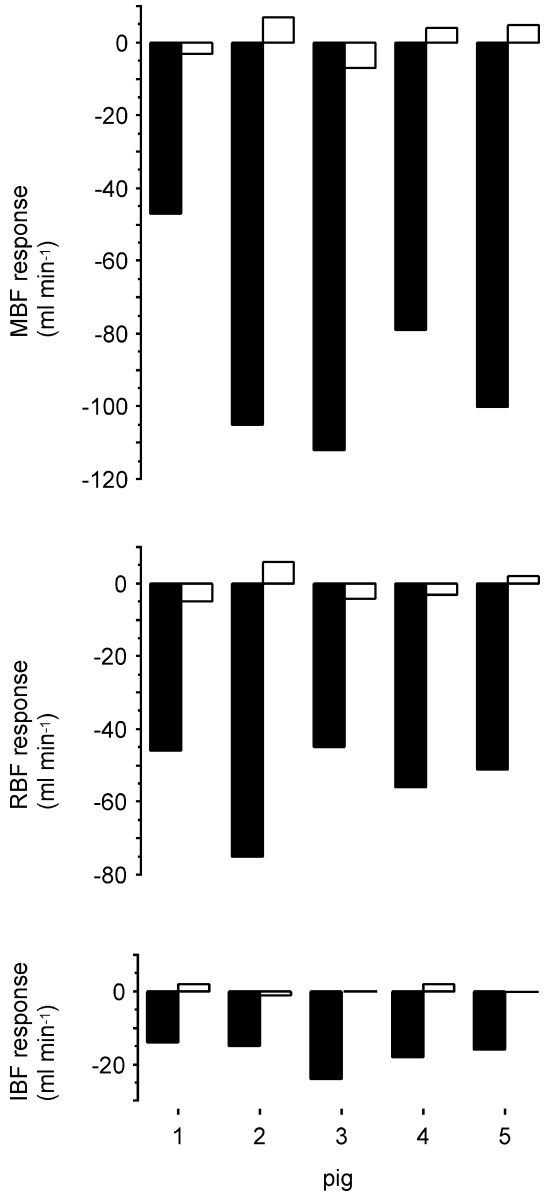

Figure 7. The effect of intra-arterial injection of l-NAME on responses of mean MBF, mean RBF and mean IBF to the intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone
Dehydroepiandrosterone was infused before (filled columns) and after (open columns) intra-arterial injection of L-NAME in five pigs (numbered 1–5).

The design of the present study has ensured that the decreases in mesenteric, renal and iliac blood flow observed during infusion of dehydroepiandrosterone were attributable to the hormone and not to other confounding factors. This was achieved through the use of a controlled anaesthetized pig preparation. Thus, prevention of changes in heart rate and arterial blood pressure and the absence of significant changes in right atrial pressure excluded any secondary interference from reflex and local physical effects. Moreover, infusion of the vehicle at the same rate as that of dehydroepiandrosterone did not reproduce any of the effects of infused hormone. Although anaesthetic agents may have vascular effects, the observed dehydroepiandrosterone-induced responses were consistent, despite the use of vasoactive agents. Furthermore, the direct relationship between dehydroepiandrosterone and its mesenteric, renal and iliac responses was further confirmed during the dose–response study, when the decreases in regional blood flow could be augmented by increasing the dose of the infused hormone. Finally, our results were not accompanied by changes in the serum levels of other active steroid hormones, which are metabolically related to dehydroepiandrosterone. Therefore, the present investigation showed that infusion of dehydroepiandrosterone to achieve serum levels which have been found in adult humans (Parker, 1999) primarily caused mesenteric, renal and iliac vasoconstriction, since these regional vascular responses did not involve changes in other haemodynamic variables. The present findings, together with previous findings on the coronary effects of the hormone (Molinari *et al.* 2003), indicated that dehydroepiandrosterone causes a widespread vasoconstriction.

Although there are data suggesting that there may be a difference in the effects of dehydroepiandrosterone on vascular disease in males and females (e.g. Barrett-Connor *et al.* 1986; Barrett-Connor & Goodman-Gruen, 1995*a*), the present findings indicate that the mesenteric, renal and iliac vasoconstriction caused by dehydroepiandrosterone was the same in male and female pigs. It is also known that plasma levels of the hormone are lower in women than in men and to decrease with age in both sexes (Barrett-Connor & Goodman-Gruen, 1995*b*; Johannes *et al.* 1999). However, the present investigation was carried out in prepubertal pigs of both sexes and showed similar basal levels of dehydroepiandrosterone

and similar dehydroepiandrosterone-induced regional vasoconstriction. Nevertheless, we cannot exclude the possibility that the vascular effects of dehydroepiandrosterone could be different in adult pigs, at least in relation to the differing levels of hormones that are influenced by gender and age.

As in our previous report (Molinari *et al.* 2003), potential mechanisms underlying the dehydroepiandrosterone-induced vasoconstriction were examined by repeating the experiment after giving vasoactive agents with underlying adrenoceptor effects. We used phentolamine to block α-adrenoceptors. The dose used in the present study has been shown in the same experimental model to abolish the reflex splenic, mesenteric, renal and iliac vasoconstriction caused by distension of the stomach (Vacca *et al.* 1996) and has been previously used to block mesenteric, renal and iliac α-adrenoceptors (Vacca *et al.* 1999; Molinari *et al.* 2002). A similar dose of the blocking agent has been used in anaesthetized pigs by other authors (Gregory & Wotton, 1981). We found that infusion of dehydroepiandrosterone after the administration of phentolamine did not affect the magnitude of responses, indicating that the regional vasoconstriction caused by the hormone did not involve α-adrenoceptors. Butoxamine was used for the blockade of β_2-adrenoceptors. The dose of 2.5 mg kg^{-1} has been shown in anaesthetized pigs to abolish the increase in arterial blood pressure and the coronary vasoconstriction caused by growth hormone (Vacca *et al.* 1998) and has been previously used in the same experimental model to block mesenteric, renal and iliac β_2-adrenoceptors (Vacca *et al.* 1996). Butoxamine completely abolished the dehydroepiandrosterone-induced vasoconstriction, indicating that the mesenteric, renal and iliac vasoconstriction involved β_2-adrenergic sympathetic effects. This result was obtained even after reversing the butoxamine-induced increase in regional vascular resistance with papaverine, indicating that baseline variables did not confound our results. In addition, the infusion of 1 mg h^{-1} of dehydroepiandrosterone reduced by about 50% the mesenteric, renal and iliac vasodilatation caused by infusion of 0.05 μg kg^{-1} min^{-1} of isoproterenol, a dose which has been previously used to examine postnatal changes in left ventricular systolic function and ventricular–vascular coupling in piglets (Cassidy *et al.* 1997). The findings that butoxamine caused vasoconstriction and completely abolished the response to dehydroepiandrosterone and that dehydroepiandrosterone significantly reduced the regional vasodilatation caused by a β-adrenergic agent suggest that the hormone caused mesenteric, renal and iliac vasoconstriction by blocking a tonic vasodilatory effect mediated by β_2-adrenoceptors. These considerations are consistent with previously reported effects of dehydroepiandrosterone on the coronary circulation (Molinari *et al.* 2003), as well as with reports showing a tonic β_2-adrenergic receptor-mediated vasodilatation in the mesenteric, renal and iliac vascular beds of anaesthetized pigs (Vacca *et al.* 1996). Previously reported findings in anaesthetized pigs have shown that growth hormone caused coronary vasoconstriction (Vacca *et al.* 1998). Although this hormone is completely different from dehydroepiandrosterone, its coronary effect had a similar underlying mechanism involving block of tonic vasodilatation mediated by β_2-adrenoceptors (Vacca *et al.* 1998).

Another mechanistic finding of the present investigation was the demonstration of the possibility that endothelial release of nitric oxide was involved in the blocking effect of dehydroepiandrosterone on tonic β_2-adrenergic receptor-mediated regional vasodilatation. Although the actual release of nitric oxide was not measured, the regional vasoconstriction induced by infusing the hormone was abolished by the local administration of L-NAME, which is known to inhibit the formation of nitric oxide (Henderson, 1991). The dose of the blocking agent used -2 mg for 1 ml min^{-1} of measured blood flow – has been previously shown in anaesthetized pigs to abolish the mesenteric, renal and iliac vasodilatation caused by intravenous infusion of 17β-oestradiol or progesterone (Vacca *et al.* 1999; Molinari *et al.* 2001*b*) and by intra-arterial infusion of testosterone (Molinari *et al.* 2002). In the present investigation, the blocking effect of L-NAME was local to the vascular bed examined, since it did not affect the dehydroepiandrosterone-induced vasoconstriction in the other vascular beds. As in the case of butoxamine, the increases in regional vascular resistance elicited by intra-arterial injection of L-NAME did not affect the results obtained with the subsequent infusion of dehydroepiandrosterone. Infusion of the hormone did not cause changes in measured blood flows, even when increases in regional vascular resistance were reversed by papaverine. The present findings are consistent with previous evidence showing in anaesthetized pigs that the tonic coronary β-adrenergic receptor-mediated vasodilatation blocked by dehydroepiandrosterone involved the endothelial release of nitric oxide (Molinari *et al.* 2003) and with previously reported findings on the role of the release of endothelial nitric oxide in the modulation or mediation of β-adrenergic effects in the coronary and peripheral

vasculature (Rubanyi & Vanhoutte, 1985; Young & Vatner, 1986; Parent *et al.* 1993; Quillen *et al.* 1992; Di Carlo *et al.* 1995).

Considering the present findings together with those previously reported on dehydroepiandrosterone-induced coronary vasoconstriction (Molinari *et al.* 2003), makes it possible to suggest a role for this hormone in the control of regional circulation. Although the results were obtained from acute experiments, they are the only available information characterizing the regional nature of dehydroepiandrosterone-induced vasoconstriction. Thus, we have shown that dehydroepiandrosterone is one of the vasoactive steroid sex hormones, with the others being 17β-oestradiol, progesterone and testosterone (Vacca *et al.* 1999; Molinari *et al.* 2001*a*,*b*, 2002). Of these hormones, only dehydroepiandrosterone caused vasoconstriction. Therefore, it is possible to propose that the vascular effect of dehydroepiandrosterone may balance the effect of the other sex hormones, as has already been previously surmised from different investigations (Ebeling & Koivisto, 1994; Barrett-Connor & Goodman-Gruen, 1995*a*; Mino *et al.* 2002; Saruc *et al.* 2003). This proposition is supported by the fact that in our investigations all these hormones caused widespread vascular effects. Also, it is remarkable that the vasoconstrictive effect of dehydroepiandrosterone is greatest in the coronary circulation, where it could oppose the vasodilating effects of 17β-oestradiol, progesterone and testosterone, which are also greatest in the coronary circulation.

A further implication of our findings is the possibility that this hormone may be involved in pathological conditions related to its vasoconstrictive effects, taking into account existing knowledge of gender- and age-related variations of its plasma levels. For instance, undue vasoconstriction has been linked to reduced insulin sensitivity, atherosclerosis and hypertension (Julius & Nesbitt, 1998). An unduly high level of dehydroepiandrosterone in older men and women has been associated with a greater arterial pressure (Hautanen *et al.* 1994; Barrett-Connor & Goodman-Gruen, 1995*a*,*b*). Increased levels in women have also been associated with insulin resistance (Ebeling & Koivisto, 1994; Mino *et al.* 2002; Saruc *et al.* 2003) and with increased cardiovascular risk (Johannes *et al.* 1999).

In conclusion, the present study has shown that intravenous infusion of dehydroepiandrosterone causes mesenteric, renal and iliac vasoconstriction. The mechanisms of this effect were shown to involve the inhibition of a tonic mesenteric, renal and iliac β_2-adrenergic receptor-mediated effect possibly related to the release of nitric oxide.

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